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Design and Synthesis of Novel Triarylethylenes as Proteasomal Inhibitors and Anti-cancer Agents

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INTRODUCTION & AIM

Protein homeostasis or proteostasis is achieved through a dynamic balance between protein synthesis and degradation.[1] The ubiquitin proteasome pathway is responsible for the breakdown of approximately 80% of intracellular proteins. The protein is firstly tagged by ubiquitin molecules, then targeted for degradation by the proteasome **(Figure 1)**.[2] Abnormalities in protein synthesis, folding, transportation, degradation, chromosomal imbalance, oncogenic activation and translation errors can lead to an imbalance in the protein homeostasis and result in a wide range of human illnesses and diseases, such as cancer, autoimmune diseases, and neurodegenerative disorders.[3]

Proteasome inhibition has emerged as a powerful strategy for anti-cancer therapy. The constitutive proteasome, commonly referred to as the 26S proteasome, is a large, multicatalytic enzyme complex of approximately 2.5 MDa, consisting of a 20S catalytic core particle made up of seven α subunits (α 1-7) and seven β subunits (β 1-7), with catalytic protease activity residing in the β1, β2, and β5 subunits. The 20S core is associated with one or two regulatory particles, which could be 19S (PA700), that is ATP-dependent, as well as 11S (PA28, PA26, REG) or PA200 which are ATP independent.[2]

Proteasome inhibitors mediate their apoptotic effect in tumor cells via inhibition of NFκB activity, inhibition of angiogenesis and DNA repair, altered degradation of cell cycle related proteins, altered pro-apoptotic and anti- apoptotic protein balance and endoplasmic reticulum stress.[2]

Ridaifen (RID) compounds are tamoxifen (TAM) analogues, with RID-F featuring two homopiperidine moieties on rings **B** and **C**. RID-F was the most effective in inhibiting all three proteasome functions which are chymotrypsin-like (CT-L), trypsin-like (T-L), and caspase-peptidyl glutamyl peptide hydrolase (PGPH).[4]

Besides the established role of proteasome inhibitor (PI) drugs in the treatment of hematological malignancies such as multiple myeloma and mantle cell lymphoma, PIs have emerged as promising drug candidates for other conditions, including neurodegenerative disorders, inflammation, immune diseases, ischemic stroke, and tuberculosis.[5]

In this light, we report the design and synthesis of novel ridaifen analogues that target the proteasome enzyme. The synthesized analogues maintained the triphenylethylene scaffold of ridaifen, while varying the substituents on rings **A**, **B** and **C**.

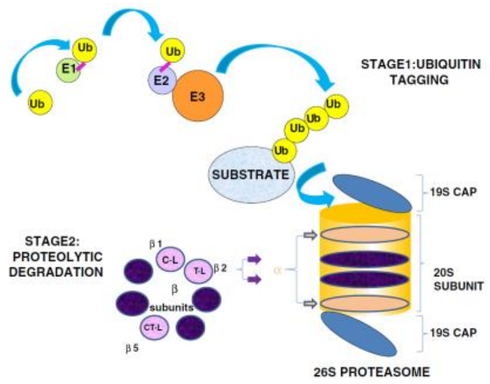
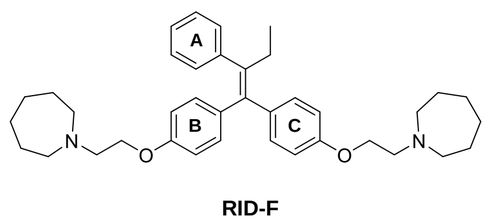


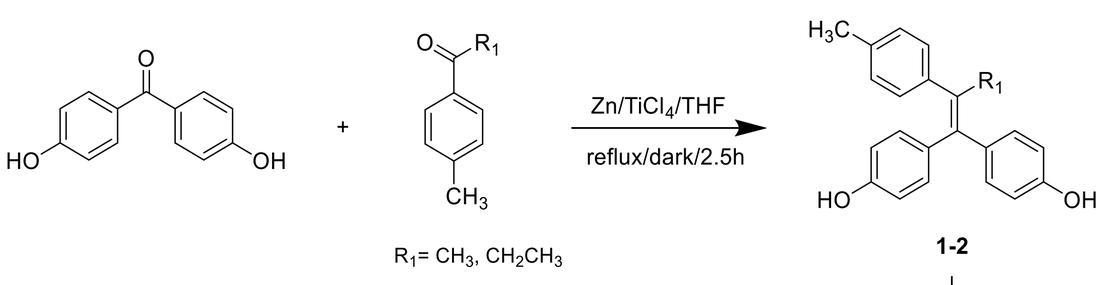
Figure 1: The Ubiquitin Proteasome Pathway [2]

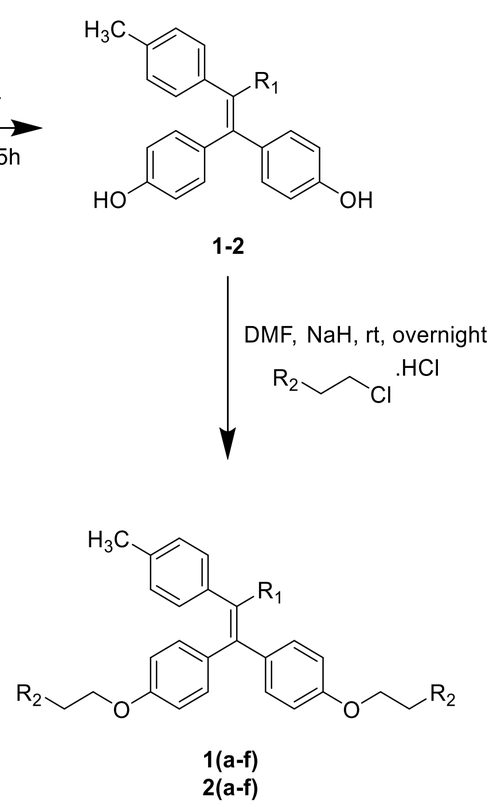


RID-F

METHOD

Synthetic scheme:





1(a-f)
2(a-f)

Biological Evaluation:

- Compounds were tested for their anti-proliferative activity by the NCI (National Cancer Institute) over 60 cancer cell lines at a dose of 10 µM. Compounds with mean growth inhibition of more than 50% were submitted for five-dose screening and their GI_{50} value was calculated.
- Compounds were biologically evaluated for their ability to inhibit CT-L catalytic subunit of the proteasome enzyme. The IC_{50} value, representing the concentration required to inhibit 50% of the enzymatic activity, was determined for each of the tested compounds.

RESULTS & DISCUSSION

Table 1: Mean GI_{50} (µM) on NCI-60 panel and inhibition of the CT-L (µM) proteasome activity.

Compound	R_1	R_2	Mean GI_{50} (µM)	IC_{50} (µM) CT-L activity
1a	CH_3	piperidin-1-yl	2.19	0.76
1b	CH_3	$-N(CH_2CH_3)_2$	1.38	**0.53**
1c	CH_3	$-CH_2-N(CH_3)_2$	ND	0.72
1d	CH_3	pyrrolidin-1-yl	**0.78**	0.93
1e	CH_3	azepan-1-yl	ND	1.71
1f	CH_3	$-N(CH_3)_2$	**0.93**	0.92
2a	CH_2CH_3	piperidin-1-yl	3.09	0.71
2b	CH_2CH_3	$-N(CH_2CH_3)_2$	**0.62**	0.72
2c	CH_2CH_3	$-CH_2-N(CH_3)_2$	**0.81**	0.89
2d	CH_2CH_3	pyrrolidin-1-yl	1.58	**0.50**
2e	CH_2CH_3	azepan-1-yl	1.70	**0.57**
2f	CH_2CH_3	$-N(CH_3)_2$	2.14	1.39
RID-F	**CH_2CH_3**	**-azepan-1-yl**	**-**	**0.54**

❑Out of the twelve synthesized compounds, ten compounds showed mean growth inhibition ≥100% on the NCI-60 cell line and was escalated to five dose screening.

❑Compounds **1a**, **1b**, **1d**, **1f** and **2a – 2f** showed mean GI_{50} ranging from 3.09 – 0.62 µM, whereas the mean GI_{50} of TAM = 4.41 µM. Thus, all the synthesized analogues showed better activity than Tamoxifen. Compounds **1d, 1f, 2b** and **2c** have shown the highest mean growth inhibition with mean GI_{50} = 0.78, 0.93, 0.62 and 0.81 µM respectively.

❑Compounds **1b, 1c,** and **1e** bearing a terminal methyl group on the ethylene backbone are compared with their congener compounds **2b, 2c**, and **2e** which feature a terminal ethyl on the ethylene backbone. Substituting the terminal ethyl (as in RID) with a terminal methyl could possibly explain the decrease in the mean growth inhibition over the NCI-60 cancerous cell lines. It is worthy to note that the homo-substitutions on rings **B** and **C** of diethylaminoethoxy, dimethylaminopropoxy and azepanylethoxy substitutent, respectively had no effect on the growth inhibition.

❑On the other hand, when comparing compounds **1d** and **1f** to their congeners **2d** and **2f** respectively, **1d** and **1f** bearing a terminal methyl group on the ethylene backbone have both shown almost two folds increase in growth inhibition.

❑Introduction of piperidinylethoxy substituent on rings **B** and **C** on either scaffolds bearing a terminal methyl or ethyl group on the ethylene backbone lead to reduced growth inhibition on the NCI-60 cancerous cell lines.

❑Three compounds, compounds **1b**, **2d** and **2e**, having diethylaminoethoxy, pyrrolidinylethoxy and azepanylethoxy substituents on both rings **B** and **C** respectively, have shown sub micromolar IC_{50} on CT-L activity, that is almost equipotent to RID-F (0.54 µM) .

❑Compound **1e** bearing azepanylethyoxy moieties on rings **B** and **C**, has shown the worst CT-L activity, three times lower than RID-F. Moreover, this compound exhibited no anti-cancer effect. The introduction of a *para*-methyl group on the unsubstituted ring **A** of RID-F and modifying the terminal ethyl to a methyl group on the ethylene backbone may have caused this reduction in potency.

CONCLUSION

❑Compounds **1d**, **1f**, **2b** and **2c** displayed the highest anti-cancer activity in comparison to the other synthesized analogues as they showed the most potent mean GI_{50} values.

❑Compounds **1b**, **2d** and **2e** have shown sub micromolar IC_{50} on CT-L activity, almost equivalent to RID-F.

❑Compounds **1e and 2f** have failed to show significant growth inhibition on the 60 cancer cell lines. Moreover, they displayed the lowest potency of all compounds against chymotrypsin-like (CT-L) protease activity. This suggests that the observed anti-cancer effect is likely attributable to the inhibition of CT-L. Previous studies have demonstrated that selective inhibitors of CT-L exhibited anticancer properties. It is noteworthy that many of the proteasome inhibitors currently approved for clinical use are potent and specific inhibitors of CT-L activity.

FUTURE WORK / REFERENCES

❑The compounds are currently being tested for trypsin-like (T-L) and caspase-peptidyl glutamyl peptide hydrolase (PGPH).

❑The promising results obtained provide a foundation for additional structural modifications to enhance the proteasomal inhibition activity, potentially leading to novel treatments for different types of cancer, including solid tumors and possibly, neurodegenerative disorders.

1. Adams, J., The development of proteasome inhibitors as anticancer drugs. Cancer Cell 2004, 5 (5), 417-421.

2. Crawford, L. J.; Walker, B.; Irvine, A. E., Proteasome inhibitors in cancer therapy. J Cell Commun Signal 2011, 5 (2), 101-10.

3. Sherman, D. J.; Li, J., Proteasome Inhibitors: Harnessing Proteostasis to Combat Disease. Molecules 2020, 25 (3).

4. Tanaka, M.; Zhu, Y.; Shionyu, M.; Ota, N.; Shibata, N.; Watanabe, C.; Mizusawa, A.; Sasaki, R.; Mizukami, T.; Shiina, I.; Hasegawa, M., Ridaifen-F conjugated with cell-penetrating peptides inhibits intracellular proteasome activities and induces drug-resistant cell death. European Journal of Medicinal Chemistry 2018, 146, 636-650.

5. Giżyńska, M.; Witkowska, J.; Karpowicz, P.; Rostankowski, R.; Chocron, E. S.; Pickering, A. M.; Osmulski, P.; Gaczynska, M.; Jankowska, E., Proline- and Arginine-Rich Peptides as Flexible Allosteric Modulators of Human Proteasome Activity. Journal of Medicinal Chemistry 2019, 62 (1), 359-370.

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